SURFACE MODIFICATION FOR BIOCOMPATIBILITY

Contract No. NS 5-2322

Quarterly Progress Report #8

January 31, 1997

The University of Michigan

David C. Martin and K. Sue O'Shea

Quarterly Progress to:	National Institute of Health
Contract Monitor:	William Heetderks, Ph.D.
Research Contract	"Surface Modification for Biocompatibility"
Contract No.	NS 5-2322
Principal Investigators:	David C. Martin and K. Sue O'Shea
Date:	January 31, 1997

Overview

This report is a summary of our activity in the eighth quarter of the contract, corresponding to the fourth quarter of 1996. This report provides an overview of the major results to date and discusses our plans for the future. In this quarter, we have been working to evaluate (1) protein polymer film deposition, morphology, and characterization, (2) bioactivity of protein polymer films *in vitro*, and (3) bioactivity and stability of protein polymer films *in vivo*. We also describe our efforts to discuss our work in (4) external communications with the scientific community.

1. Protein Film Deposition, Morphology, and Device Characterization

In our previous work and reports we have demonstrated our ability to tailor the morphology of protein polymer films when deposited onto silicon substrates. These results have enabled us to create three distinctly different classes of protein films by changing processing conditions:

1. Cast or Dipped continuous films:

By casting a solution onto the surface of the substrate and allowing for the solvent to dry, or by dipping a substrate into the solution and retracting the sample at a controlled rate, we can deposit uniform, continuous films of polymer onto the solid substrate.

2. Discontinuous, beaded coatings

By electrodeposition of polymers from dilute solution, beaded films can be created which are composed of small droplets with typical diameters of ~100 nm. The size of the droplets can be varied by changing the electric field, solution concentration, and separation distance from the sample surface.

3. Discontinuous, fibrous coatings

By electrodeposition of the protein polymer from more concentrated solutions, fibrous mats can be created. The filaments have diameters which are also ~100 nm, and can likewise by varied with processing parameters.

4. Multicomponent films

We have also now demonstrated that the discontinous coatings can be created with blended components of a variety of small molecules including aspirin, caffeine, and antibiotics.

Mechanical Characterization

In this quarter we have made progress in characterizing the mechanical properties of these thin protein polymer films as a function of their morphology. To accomplish this goal, we have investigated the response of the coating to a point applied load using a Buehler Micromet II Digital Microhardness tester with a Vicker's indentor. The purpose of these experiments is to provide estimates of the mechanical properties of these films to help optimize performance for their eventual use in-vivo.

Enclosed are scanning electron micrographs of the three types of films: 1) dipped continous, 2) beaded discontinous, and 3) fibrous discontinous. In all three cases, the films were nominally 100 nm thick. Shown in the micrographs are the deformation responses observed after (1) 500 gf load (Figure A-1), and (2) 100 gf load (Figure A-2).

The micrographs show dramatic changes in plastic deformation response as a function of film morphology. The continous polymer coatings show extensive plastic deformation which outlines the shape of the pyramidal Vicker's indentor. The beaded coating spalls off the substrate, and there is little evidence for plastic response in the film itself. The fibrous coating shows evidence for flattening directly under the indentor, but again little or no evidence for permanent distortion well away from the point of indentation itself.

We are currently comparing this experimental data to models of the elastic and plastic deformation response of thin polymer films on solid substrates. The ability to control the morphology allows use to examine the manner in which stress transfer is accomodated. The utility of local indentation for examining the adhesion of polymer films on solid substrates is a topic of current research interest. Recent work by Ritter et al. (1992) has shown that quantitiative analysis of the deformation response of polymer films on substrates can provide information about the adhesion of the film (Figure A-3). We are currently evaluating the depth of the indentation, the volume and size of the coating pile-up and comparing these results to available theoretical models. In these experiments, we are invesetigating variations in the observed deformation reponse as a function of film thickness and applied load.

Electrical Characterization:

We have now established a reproducible and reliable experimental protocol for characterization of the impedance changes of the protein polymer coated probes as a function of coating time and separation distance. The impedance spectra are acquired from 1 to 10^6 Hz using a 20 mV AC signal on a EG&G instrument with a saturated calomel reference electrode and a platinum counter electrode. These experiments make it possible for us to evaluate the absolute magnitude, as well as the resistive and capacitive components of the impedance of the polymer thin films as a function of their morphology and thickness.

From these experiments, we have now obtained estimates of the increase in probe impedance as a function of coating thickness. Our results indicate that the rate of increase of impedance with thickness is higher for continous films than for discontinous films. We are currently investigating the relationship between impedance changes and film thickness quantitatively. Our best estimates to indicate that for films with nominally the same thickness, the continous coatings show an incremental increase in impedance that is twice as high as the discontinous coatings.

Included with the report are figures showing the variations in impedance response for both continuous and discontinous (fibrous) coatings. In the first graph (Figure A-4), the incremental increase in impedance amplitude above the uncoated substrate, normalized by the impedance of the uncoated substrate ($\Delta Z/Z$), is plotted as a function of frequency. It can be seen that the magnitude of the increase in impedance is larger as the film becomes thicker. Shown in the bottom panel is the magnitude of the increase in impedance at 1000 Hz, plotted as a function of film thickness. The impedance at this frequency increases with thickness. The rate of increase of impedance with thickness evidently decreases as the film gets thicker.

In the second figure (Figure A-5) the incremental change in impedance ($\Delta Z/Z$) is again shown as a function of frequency. The bottom panel shows the increase with increasing coating time. In previous work we have found that the rate of film deposition is 100-150 nm per second. These estimates are making it possible for us to construct a plot of the incremental increase in impedance for both continuous and discontinuous films as a function of thickness (Figure A-6). Further efforts are under way to confirm the variations in coating thickness using scanning electron microscopy (SEM) and atomic force microscopy (AFM).

2. Bioactivity of Protein Polymer Films *in vitro*

We have made significant progress this quarter in developing methodologies for patterning the surfaces of substrates. In the past we have reported on schemes to pattern the surfaces of devices using modifications of microlithography techniques, involving the electrodeposition onto patterned photoresist, followed by removal of the resist with a suitable solvent (acetone). We have also discussed the use of a shadow masking technique in which the proteins can be deposited onto a substrate through a grating with controlled geometry.

In the past quarter, we have been developing patterning methodologies to address questions of substrate preference of neuronal and glial cells. Leading this effort is post-doctoral research scientist Dr. Libby Louie (CV enclosed). Dr. Louie has coated coverslips with protein binding materials (nitrocellulose), and using a stamp micromachined with a stripe 150 microns wide by 600 microns in length, wicked protein containing solutions onto solid surfaces in a spoke pattern (Figure B.1). Uncoated glass, and glass coated with hydrophobic silanes then coated with protein have been used as substrates. Using antibodies to the adsorbed protein (anti-fibronectin, anti-laminin, anti-slp) she is examining the resulting pattern (Figure B.2). To date, the most accurate patterns have been produced using silane follwed by ECM protein.

While patterns produced were occasionally incomplete, the cells were able to recognize and respond to the patterned substrates succesfully. Neuro2A cells plated on silane-laminin coated coverslips spread and extended neurites on the laminin surface after two hours in vitro (Figure B.3).

Plans:

We are continuing to refine our patterning techniques to improve protein distribution and the uniformity of cell spreading. Dr. Louie has designed a number of patterns with various lane sizes, diameters, and geometries. We are also in communication with the Cornell National Nanofabrication Facility for their assistance in creating patterned grids for this purpose.

3. Bioactivity of Protein Polymer Films *in vivo*

Progress:

In the past quarter, significant progress has been made by the Kresge Hearing Research Institute in sectioning silicon probes embedded in tissue for histological examination. Rick Altschuler and Pete Finger have shown that the EXACT method of tissue sectioning enables them to make thin sections through probes, suitable for confocal optical microscopy. The technique involves a water-cooled, diamond-impregnated wire saw that facilitates the rapid sectioning through tissues with a variety of hardnesses.

A micrograph demonstrating the utility of this technique is shown in Figure B.4, in which the exquisite cellular detail of elements in the near vicinity of a probe type can be discerned.

Plans:

Now that this sample preparation protocol has been completed, we are prepared to implant protein polymer coated silicon probes for morphological examination. We are currently preparing uncoated, SLPF coated, SLPL coated, and SELP coated probes for implanation into Guinea Pig CNS. The morphological response of the coated probes will be examined and compared to our previous results on coated polypropylene suture.

Also, it has not yet been possible to specifically identify the cell types near the probe tip. Currently, the tissue block containing the implant has been incubated in propidium iodide (which stains nuclei), then embedded and sectioned. Unfortunately, even using confocal microscopy we have not been able to identify neurons and glial cells based solely on chromatin structure. We are now carrying out pilot studies to determine if other fluorophores and dyes may be used to more accurately identify cell types near the implant. This analysis will be crucial in determining the nature and extent of cell reaction to coated probes.

4. External Communications

A poster titled "Impedance Spectroscopy of Protein Polymer Coated Micromachined Silicon Probes" by Shenkarram Athreya, Chris Buchko, and David C. Martin, was presented at the annual Macromolecular Science and Engineering Symposium.

A poster titled "Electrostatic Deposition of Protein Polymer Blends", by Chris Buchko, Loui Chen, and David C. Martin, was presented at the annual Macromolecular Science and Engineering Symposium.

A talk titled "Impedance Spectroscopy of Protein Polymer Coated Micromachined Silicon Probes" by Shenkarram Athreya, David C. Martin, James Weiland, and David J. Anderson was presented by Shenkarram Athreya on Monday, December 2nd at the fall 1996 meeting of the Materials Research Society meeting in Boston, MA.

A talk titled "Electrostatic Deposition of Protein Polymer Blends" by Christopher J. Buchko, David C. Martin, Michael A. Johnson, and Loui Chen was presented by Christopher J. Buchko on Monday, December 2nd at the Materials Research Society in Boston, MA.

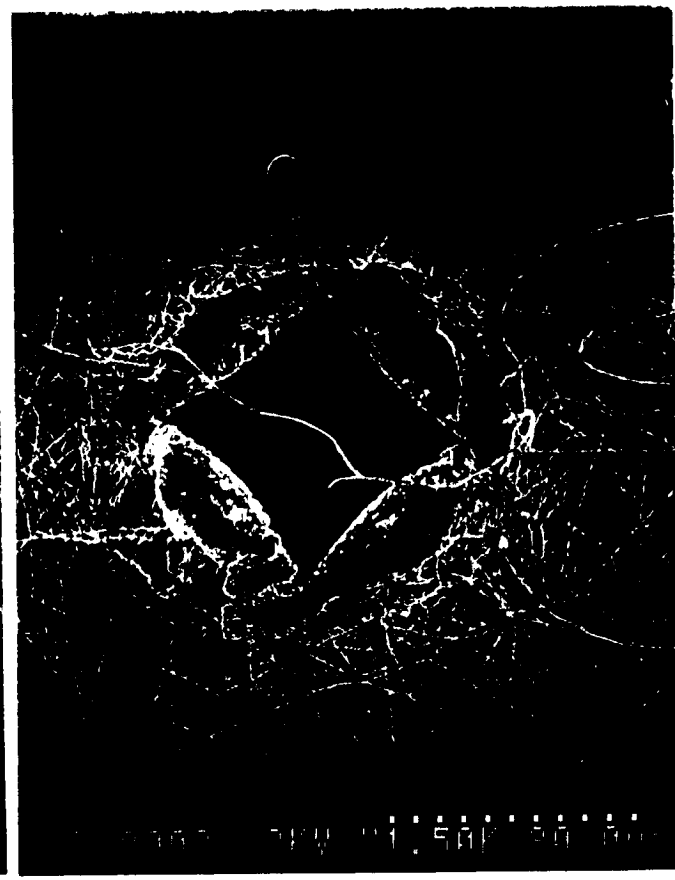
A paper titled "Electrostatic Depostion of Protein Polymer Blends" by Christopher J. Buchko, Loui Chen, and David C. Martin, was submitted for publication in the proceedings volume from the fall 1996 Materials Research Society. A copy of this document is enclosed.

A poster titled "Biocompatibility of CNS Implants Coated with Silk-llike Polymers Containing Elastin, Fibronectin, or Laminin Cell Binding Motifs", by K. S. O'Shea, C. Buchko, Y. Shen, R. A. Altschuler, P. Finger, J. A. Wiler, J. Cappello, and D. C. Martin, was presented by K. Sue O'Shea at the 1996 annual meeting of the Society for Neuroscience, November 17, 1996.

DIPPED COATING

BEADED COATING

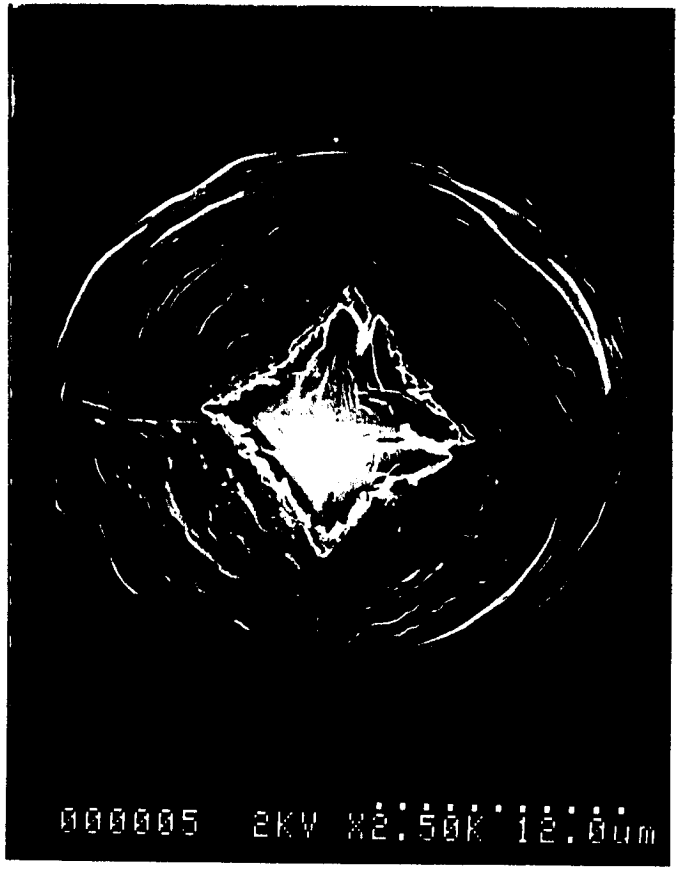
FIBROUS COATING



500 gF INDENTATIONS

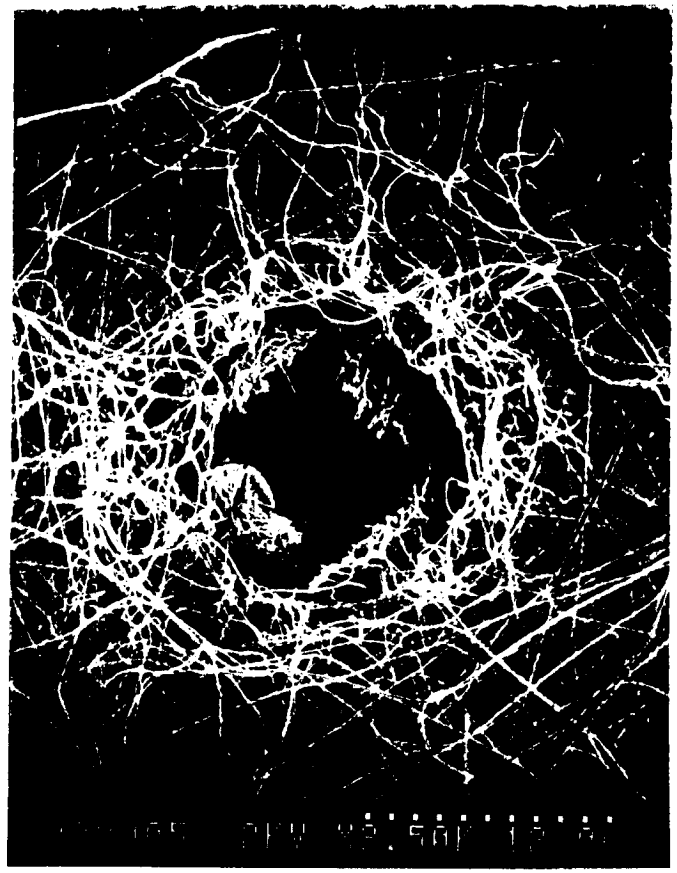
FIGURE A-1

DIPPED COATING



BEADED COATING

FIBROUS COATING



100gf INDENTATIONS

FIGURE 2

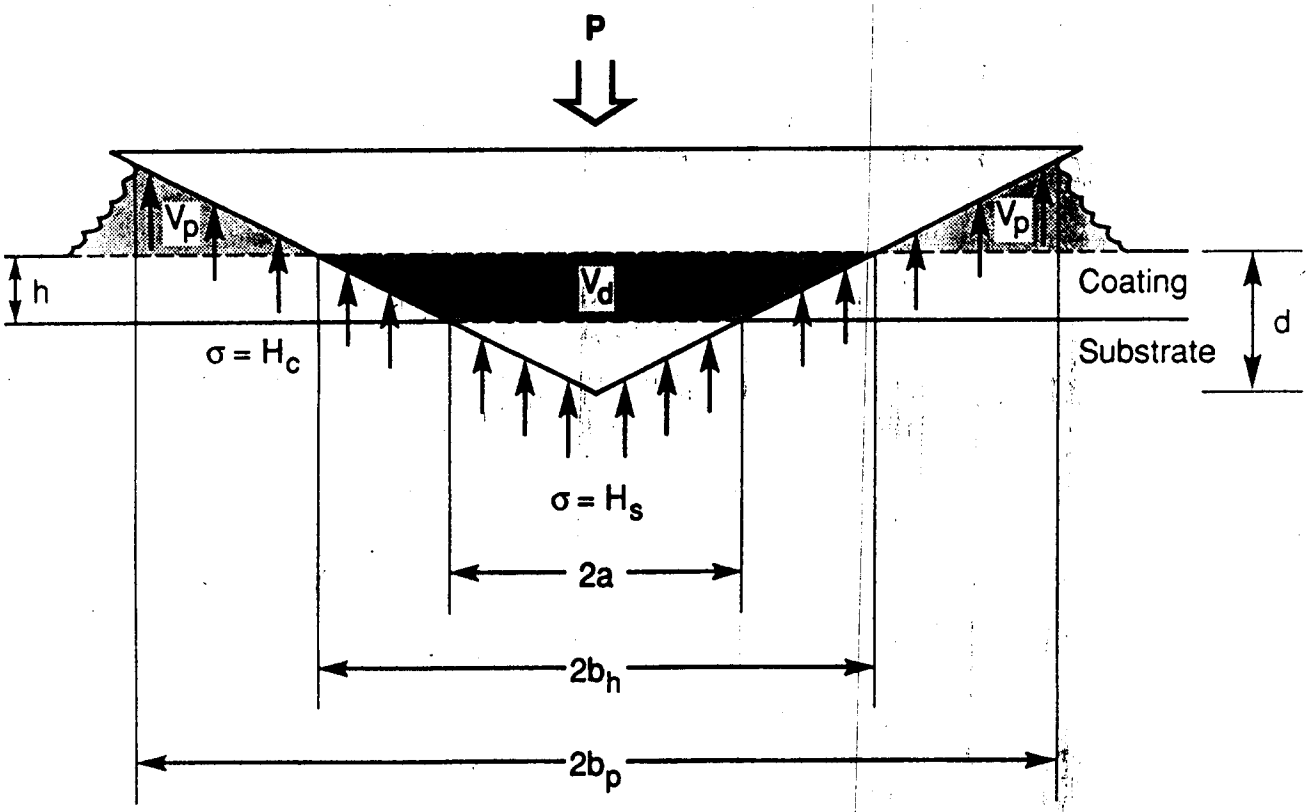
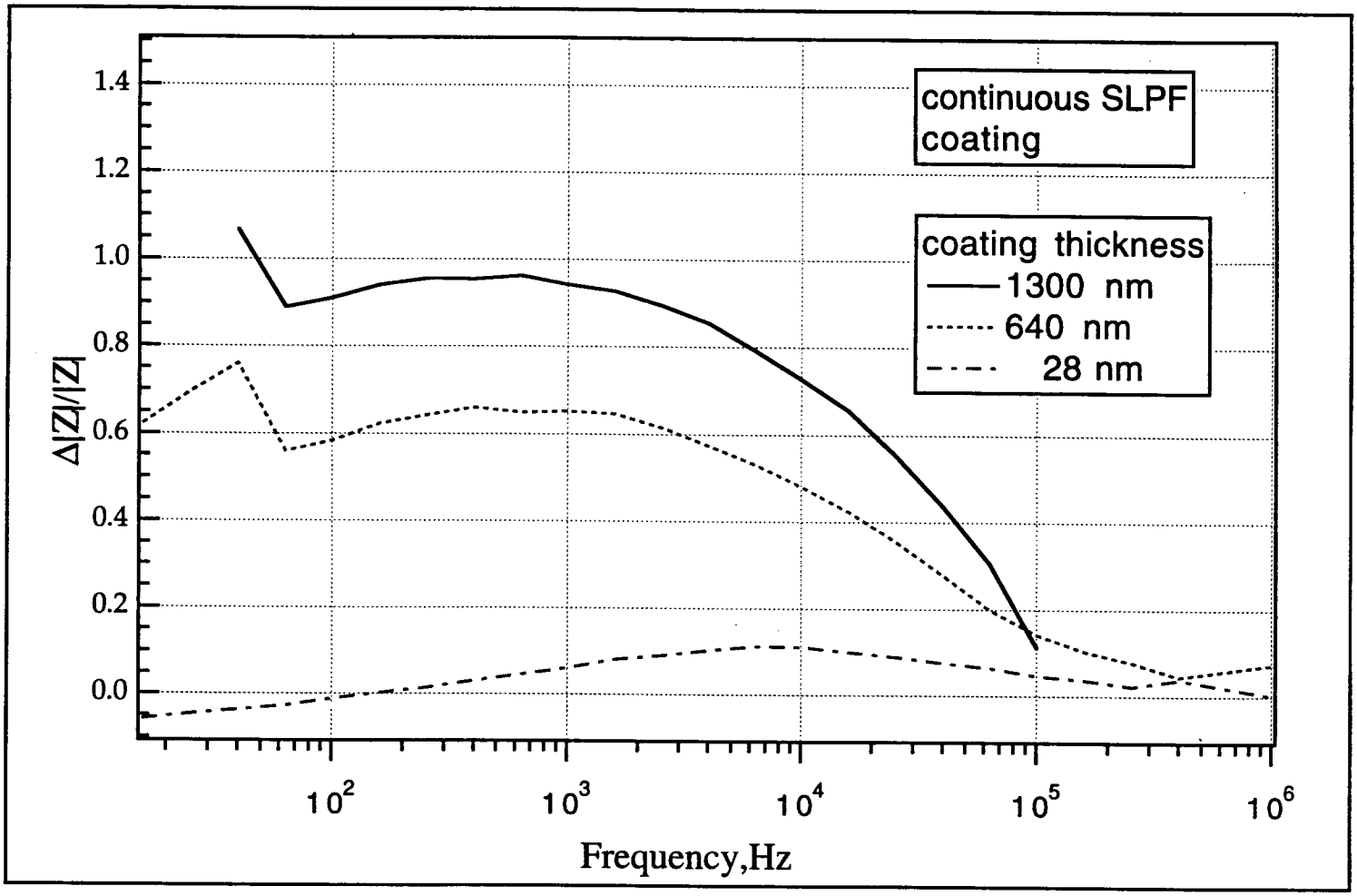


Fig. 4. A schematic of the pile-up load sharing model.

Figure 3


continuous SLPF coating
coating thickness
1300 nm
640 nm
28 nm
Δ|Z|/|Z|
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10^2
10^3
10^4
10^5
10^6
Frequency,Hz

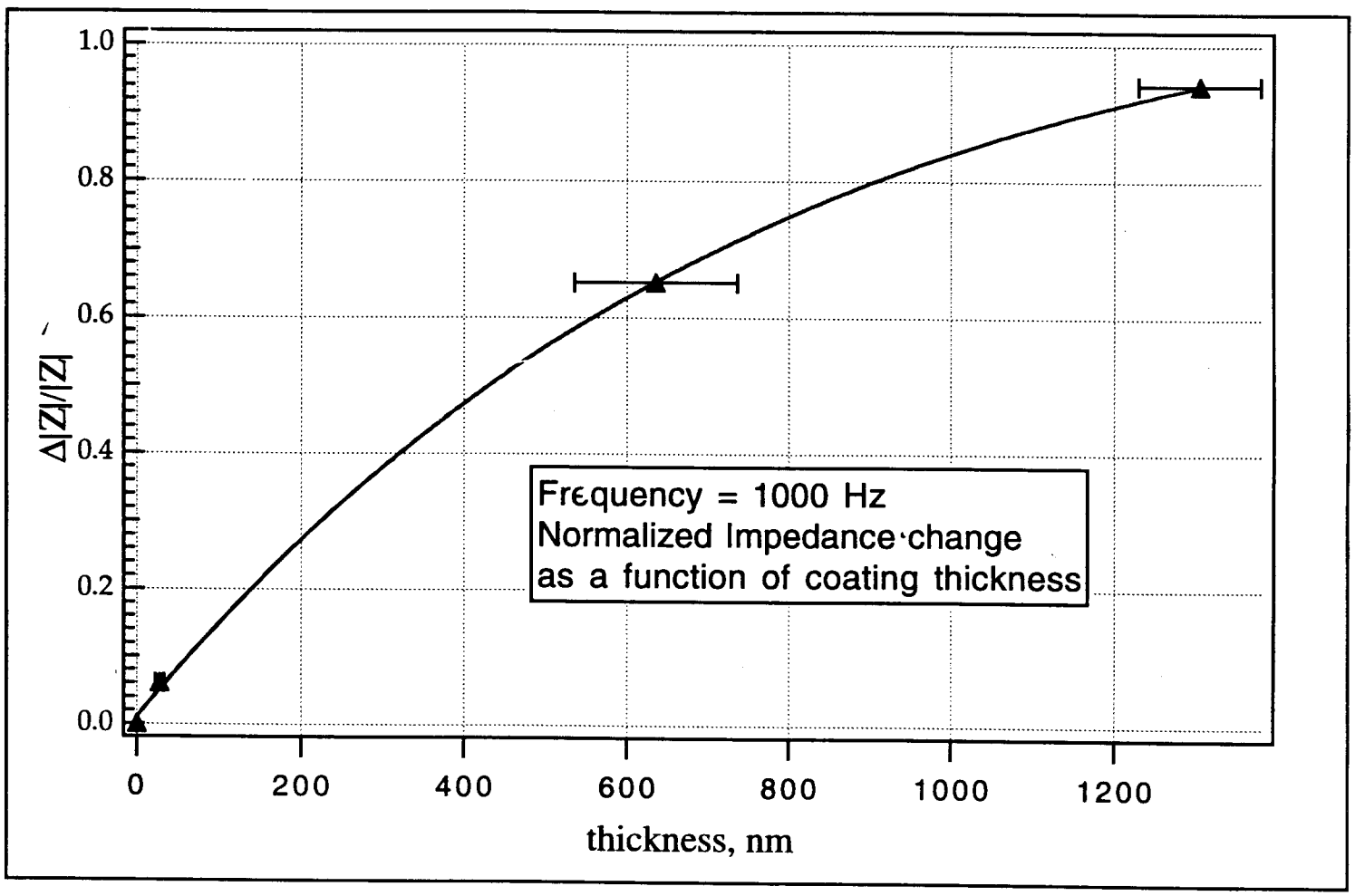
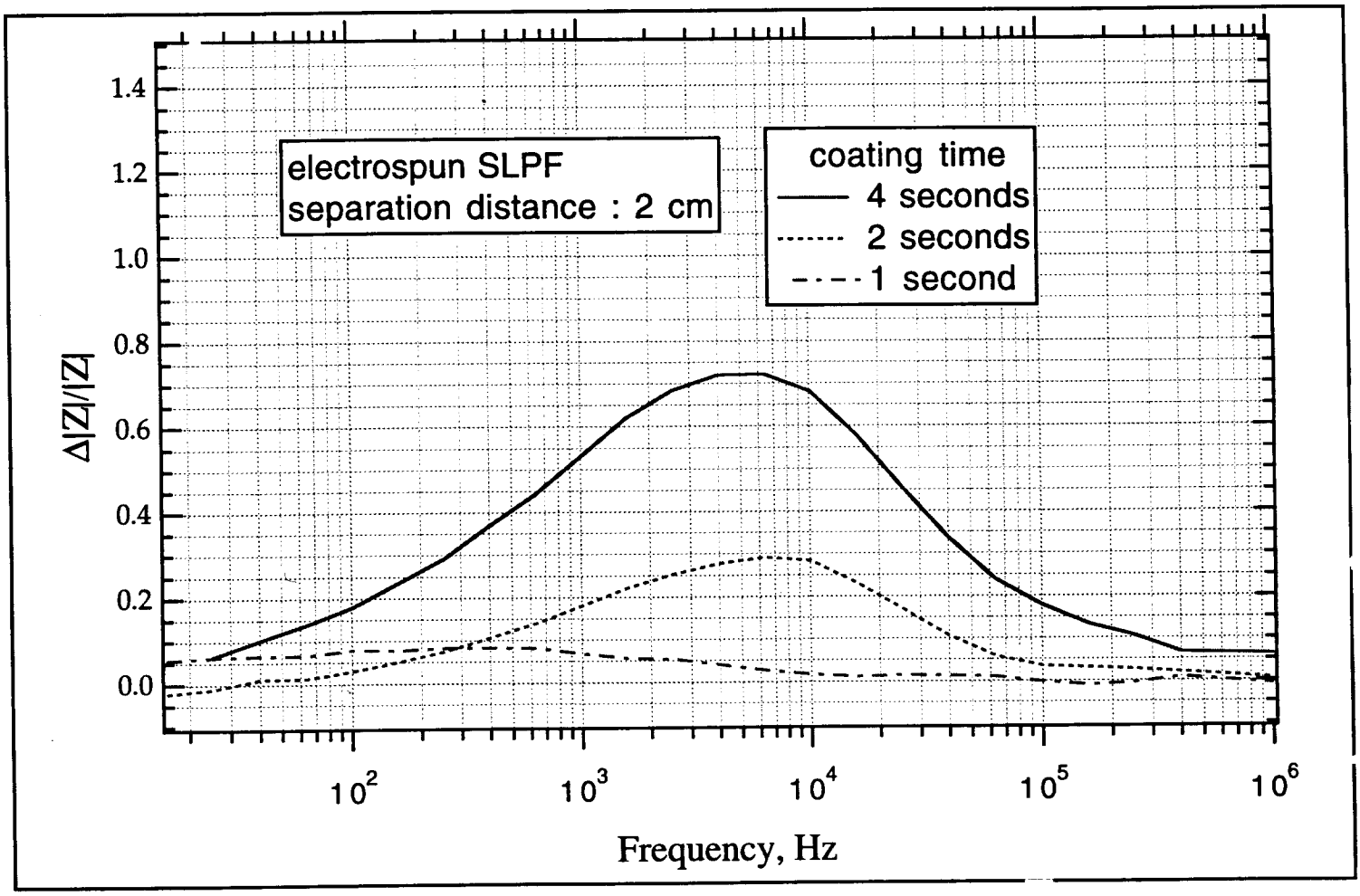

Frequency = 1000 Hz
Normalized Impedance change
as a function of coating thickness
Δ|Z|/|Z|
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
1200
thickness, nm

FIGURE A-4


electrospun SLPF
separation distance : 2 cm
coating time
4 seconds
2 seconds
1 second
Δ|Z|/|Z|
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10^2
10^3
10^4
10^5
10^6
Frequency, Hz

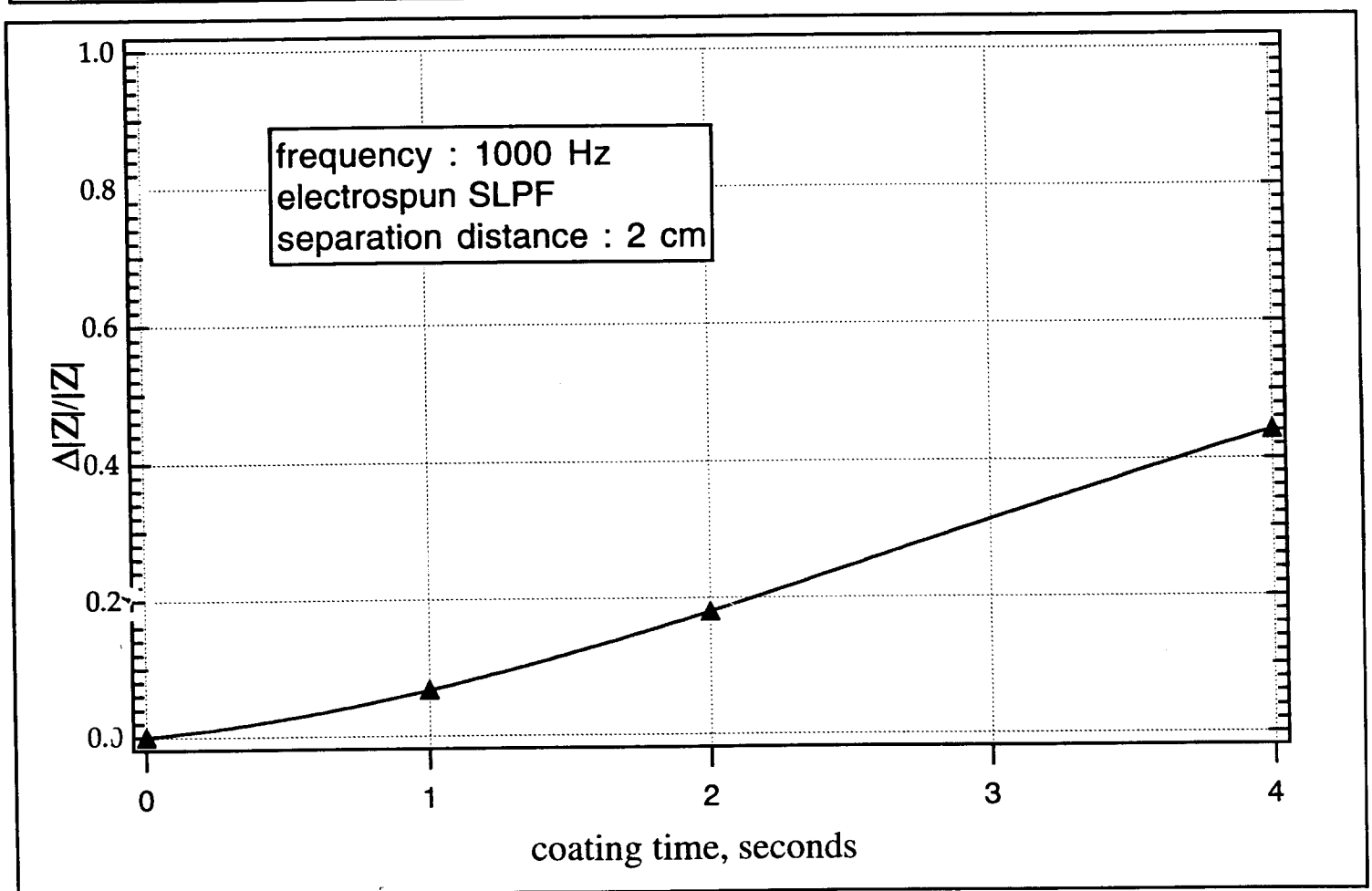

frequency : 1000 Hz
electrospun SLPF
separation distance : 2 cm
Δ|Z|/|Z|
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
coating time, seconds

FIGURE A-5

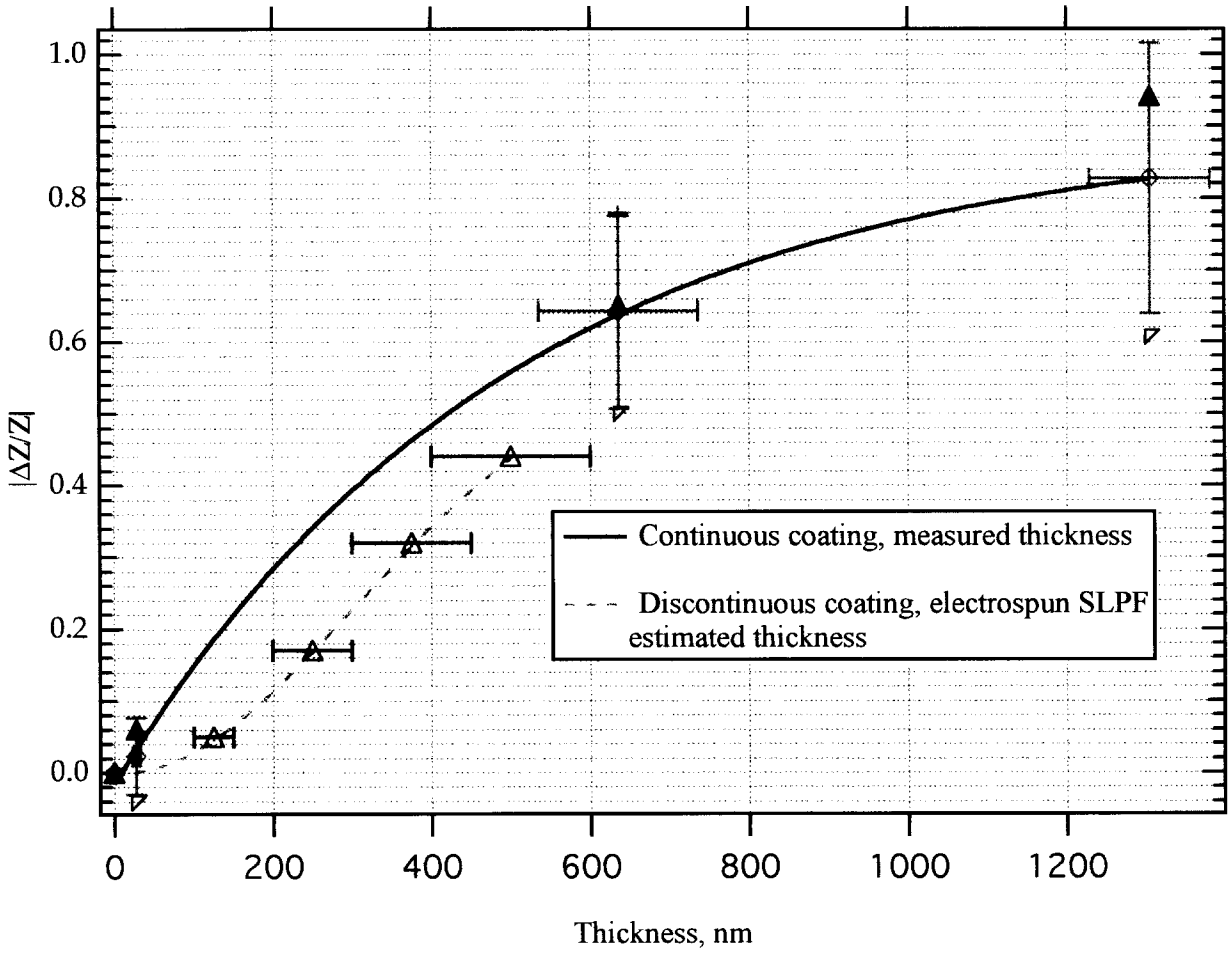

1.0
0.8
0.6
0.4
0.2
0.0
|ΔZ/Z|
0
200
400
600
800
1000
1200
Thickness, nm
Continuous coating, measured thickness
Discontinuous coating, electrospun SLPF estimated thickness

FIGURE A-6

FIGURE LEGENDS

B.1 Spoke pattern produced by stamping protein onto coverslips.

B.2 Indirect immunofluorescence image illustrating the sharp edge of the laminin stripe. Coverslips were coated with silane followed by 10 ug/ml laminin for 60 minutes at 37^{o}, then rinsed, incubated in anti-laminin antibody, followed by goat anti-rabbit IgG - FITC.

B.3. Neuro2A cells (approx. 1 x 10^{5} cells/ml) were cultured for 2 h on coverslips treated with silane followed by stripes of laminin. Cells were clearly able to distinguish the edges of coated coverslip.

B.4. Stacked confocal image (montage of 13 sections) illustrating the cell response to the implant after 3 weeks. Nuclei were stained with propidium iodide prior to embedding, then sectioned, and examined using our Biorad confocal microscope.

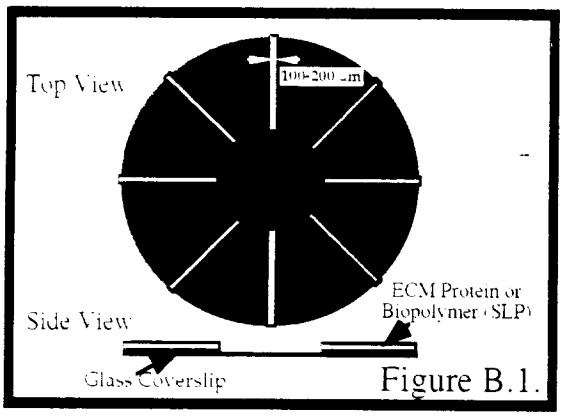


Figure B.1.

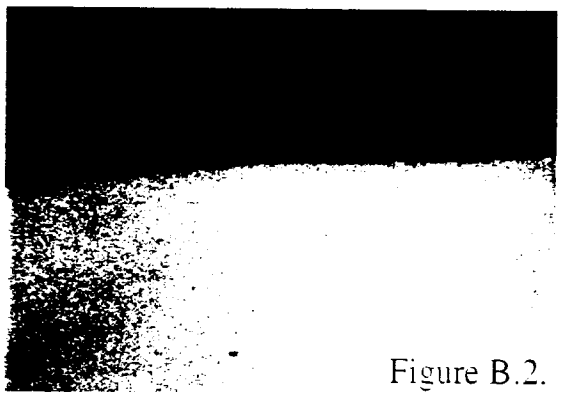


Figure B.2.

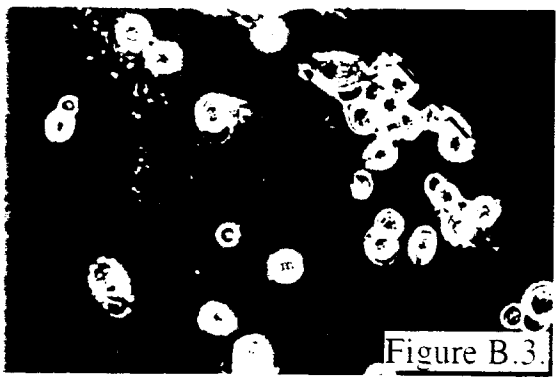


Figure B.3.

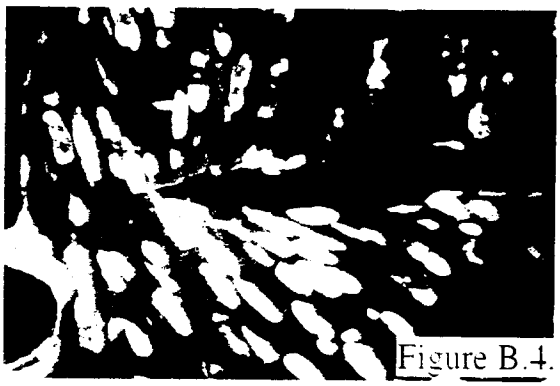


Figure B.4.

ELECTROSTATIC DEPOSITION OF PROTEIN POLYMER BLENDS

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ABSTRACT

The feasibility of electric field mediated deposition of blends of small molecules and protein polymers is presented. Protein polymer coatings are being investigated for use as surface modifiers for biocompatibility. The inclusion of small molecules in these coatings could help promote favorable biological responses. The influence of these molecules on the morphology of the coatings is examined by Scanning Electron Microscopy, Transmission Electron Microscopy, and Wide Angle X-ray Scattering. Small molecules soluble in the processing solvent are found to blend well into the protein polymer coatings.

INTRODUCTION

Electric field mediated deposition of protein polymers creates coatings and thin films that can be used to modify the surface of prosthetic devices to improve the biocompatibility of these devices. The prosthetic devices used in this application were micromachined silicon devices fabricated at the Center for Neural Communication Technology at the University of Michigan [1]. These devices were designed to be implanted in the peripheral and central nervous system in cases of chronic nerve damage. Engineering the device/tissue interface places the following requirements on a biocompatible coating:

(1) to provide an adhesive surface for specific cell types,
(2) to facilitate the integration of the device into tissue,
(3) to mediate the large difference in mechanical properties between the stiff device and the soft tissue,
(4) to encourage neurite outgrowth to the stimulating/recording sites on the device,
(5) to prevent unfavorable biological reactions, such as gliosis, infection, etc., and
(6) to minimize the impedance of signal conduction from neurons to the stimulating/recording sites.

Electric field mediated deposition (electrospinning) can meet some of these requirements (1, 2, 3 and 6) by producing a discontinuous, porous, high surface area coating composed of submicron polymer filaments [2].

The fibrous polymer coating produced by electrospinning is composed of a genetically-engineered protein polymer designed to be a powerful cellular adhesive [3]. The recognition elements included in the backbone of this polypeptide are domains selected from extracellular matrix proteins known to bind to certain cell types. In addition, there are silk-like segments in the backbone that provide structural stability and processability. The protein we use most frequently is a silk-like polymer with fibronectin binding functionality (SLPF) [4]. This combination of design elements allows for a variety of processing schemes, producing a wide range of solid forms, including thin films, fibers, and membranes [5]. These products can display mechanical strength and cellular adhesion. However, the binding domains contained in the backbone of the molecule may not be sufficient to generate neurite outgrowth (requirement 4) and prevent certain cellular responses (requirement 5). Some cellular responses, like gliosis, may also display a time dependent behavior so that preventative measures may need to be only temporary [6]. We are investigating the incorporation of appropriate small molecules and proteins in the polymer coating for delivery to the implant site, and for release over time.

Polymeric materials are familiar carriers for *in vivo* drug delivery. Methods for producing implantable delivery devices have been well-documented and continue to be a source of much investigation [7]. These materials often release their blended components by eroding [8] or by a

Appearing in *Enabling Biomaterials Technologies - Molecular Structure-Properties Relationships*, ed. by Anuj Bellare, Myron Spector, David Williams (Materials Research Society Proceedings, Pittsburg, PA 1997)

response to a chemical or physical change [9]. In most cases, the polymeric carrier is the entire implant, rather than a component of or a coating on another implant. This study examines the feasibility of coprocessing small molecules and proteins with SLPF, paying specific attention to the microstructure of the resulting coating. Given the desirable properties that the electrospun polymer coating has demonstrated and the relationship these properties have to coating morphology, it is essential to determine if drugs can be effectively delivered by the fibrous coating while it retains these properties. Precedent for this exists from the study of electrospun polymer coatings as wound dressings and pesticide carriers[10]. It was postulated that size, weight percent, and solubility of the secondary component in formic acid (the processing solvent) and in SLPF would affect the morphology of the coating and therefore the release of the drug.

METHODS

SLPF (Mw 76 kD) and Nylon 6,6 (Mw ~ 50 kD) (chosen for its rheological similarity to SLPF) were dissolved at 10.5% and 15%, respectively, by weight in formic acid. A variety of small molecules (Mw 100-4000) was tested for solubility in formic acid, resulting in aspirin, caffeine, and kanamycin (in addition to other antibiotics) being chosen for this study as model small molecules with some biological activity (Table 1). The polymer and small molecule solutions were mixed in various ratios prior to deposition. In some cases, the solid materials were mixed together before being dissolved in formic acid.

The electrospinning apparatus consists of a syringe containing the polymer solution, a needle (152 μm O.D.), and a target held at ground (typically a diced piece of a silicon wafer) The polymer solution is given a negative bias (3 kV-10 kV) by means of an electrode embedded in the syringe, and the resulting field between the needle and grounded target draws polymer solution out of the capillary, creating many submicron filaments. The volatile formic acid solvent evaporates as the fibers travel across a gap of 5.0 mm to 40 mm, producing a fine, filamentous web coating the silicon target.

After coating, the silicon targets were prepared for examination in the Scanning Electron Microscope (SEM) (Hitachi S-800). Some coatings were soaked in water for 1-3 days prior to SEM in order to observe the effect of small molecule release on coating morphology. Samples for Transmission Electron Microscopy (TEM) (JEOL 2000 FX) were prepared by electrospinning blended solutions onto carbon coated mica. The thin carbon film was floated off onto deionized water and lifted onto copper grids. Samples for Wide Angle X-Ray Scattering (WAXS) were prepared by electrospinning blended solutions onto silicon and glass substrates. Blended solutions were also cast onto silicon and glass substrates.

RESULTS

The first processing parameter evaluated in this study was the weight percent of secondary component and its influence on coating morphology. Figure 1 is a SEM micrograph of 11% by weight caffeine blended with nylon and electrospun onto a silicon wafer. Caffeine is not soluble in formic acid (the processing solvent), and therefore does not readily mix with the nylon in solution. Caffeine particles appear as large, irregular clumps in the fiber. Some are suspended in the fibers, and some are found separate from the fibers on the silicon surface. Higher weight percents of caffeine caused less fiber formation, and at 50% no fibers were formed. Unlike caffeine, aspirin is soluble in formic acid, and experiments were performed with both nylon and SLPF. In the aspirin/nylon system, up to 50% by weight aspirin was blended before fiber formation was disrupted. This result was distinct from the caffeine result in that although fibers were not formed at 50% by weight aspirin, there were no obvious aspirin rich domains. Even at 11% by weight caffeine, there were obvious caffeine rich domains interspersed among the fibers.

A systematic examination of the morphological effects of electrospinning blends of aspirin and SLPF is found in Figure 2. This series of SEM micrographs shows the transition from fibers to droplets caused by the addition of more aspirin. While the morphology of the coating clearly changed, there was no obvious change in the texture of the surfaces, nor were there any obvious aspirin rich domains.

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Table I
Molecules Blended and Electrospun with SLPF

Aspirin	138 g/mol
Amoxicillin	365 g/mol
Bacitracin	1420 g/mol
Caffeine	194 g/mol
Cephalosporin C	415 g/mol
Erythromycin	734 g/mol
Hygromycin B	528 g/mol
Kanamycin	485 g/mol
Neomycin Sulfate	909 g/mol
Nystatin	926 g/mol
Tetracycline	444 g/mol

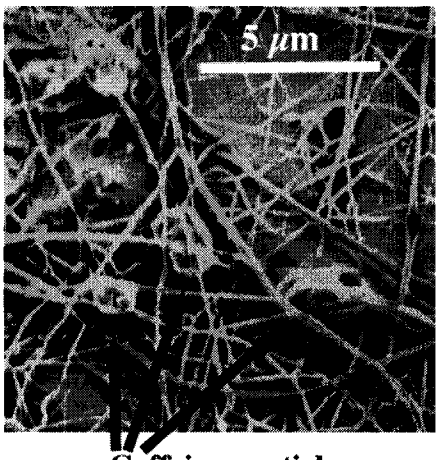

Figure 1 SEM of electrospun filaments of a 11% by weight caffeine/nylon blend.

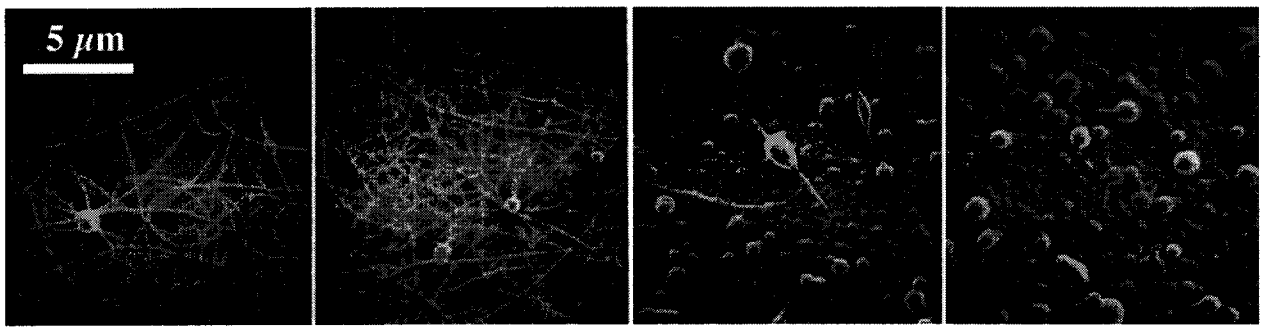

Figure 2 SEM of electrospun filaments of aspirin/SLPF blends. From left to right, the concentration of aspirin is 14%, 25%, 33%, and 50% by weight.

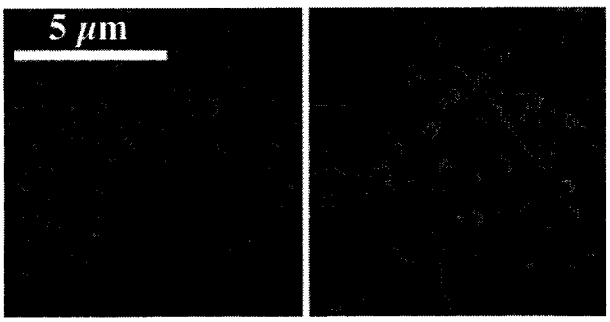

Figure 3 SEM of electrospun 33% by weight aspirin/SLPF coating. The left image is before soaking in water for 72 hours and the right image is after the soak. No morphological differences are apparent in the coating after the blended molecules diffuse out.

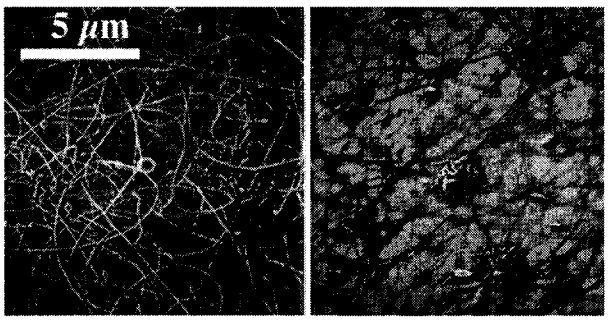

Figure 4 SEM of electrospun 25% kanamycin/aspirin blends. The left image is prior to a 72 hour soak in water and the right image is after the soak. Note the presence of pores in the right coating.

The other molecules blended with SLPF for this study were antibiotics (Table I). These molecules were all soluble in formic acid, and were mixed at 25% by weight with SLPF and electrospun onto silicon wafers. All the blends at this concentration, chosen because it was consistent with weight fractions of molecules delivered in other systems [7], produce fibers with no obvious antibiotic rich domains.

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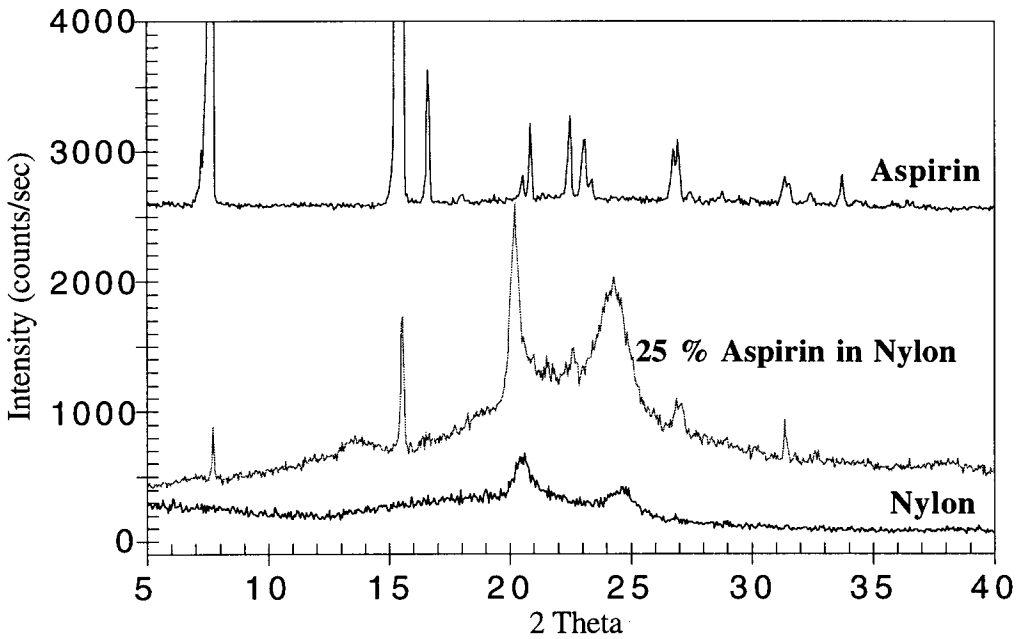

Figure 5 WAXS of cast nylon, aspirin, and nylon/aspirin blended thin films. Peaks from crystalline aspirin appear in the blended thin film, indicating phase separation.

Since these films were designed to deliver the included molecules in an aqueous system, several of the coatings were soaked in water for 24 to 72 hours and then examined in the SEM. Figure 3 shows that there is no observable difference in the aspirin/SLPF (33% by weight aspirin) coating morphology after soaking for 72 hours. Aspirin was precipitated out of the water that the sample was soaking in after the sample was removed, confirming that the small molecules were diffusing out of the coating. The effect of this diffusion on the coating morphology could not be observed in the SEM. However, in the kanamycin/SLPF system, it was observed that the coating became full of holes after a 72 hour soak in water (Figure 4).

WAXS of blended coatings was undertaken to explore the extent of mixing of the two components. Figure 5 is a WAXS of 100% aspirin, a blend of 25% aspirin by weight in nylon, and 100% nylon films cast from a formic acid solution onto a silicon substrate. The crystalline peaks of both 100% aspirin and 100% nylon are obvious in the blend. WAXS of aspirin/SLPF cast films also show peaks from crystalline aspirin along with broad scattering from the SLPF (Figure 6). Figure 7 is a WAXS of 100% aspirin, 25% aspirin by weight in nylon, and 100% nylon films electrospun onto a silicon substrate. In this instance, the blend does not contain the peaks from crystalline aspirin.

In order to further identify the presence of small molecule rich domains inside electrospun fibers, samples were examined in the TEM by Bright Field, Dark Field, and Selected Area Electron Diffraction. Although crystallites of aspirin were identified in 100% aspirin electrospun from formic acid, no aspirin crystallites or crystalline aspirin diffraction was apparent in electrospun aspirin/SLPF (25% by weight) samples. Bright field images showed morphological similarity between electrospun SLPF fibers and 25% by weight aspirin/SLPF blended fibers.

DISCUSSION

The results above indicate that it was feasible to electrospin fibers from blends of small molecules with SLPF whose morphology was essentially consistent with unblended SLPF. However, specific process parameters influenced the morphology of the resulting thin films. The first of these parameters was solubility in formic acid. Caffeine was not incorporated as homogeneously with the polymer in the coating as aspirin. There were significant deposits of caffeine among the polymer fibers well below the threshold concentration for fiber spinning. The insolubility of caffeine in formic acid suggests that a significant fraction of it would immediately dissolve away when the coating was placed in an aqueous environment.

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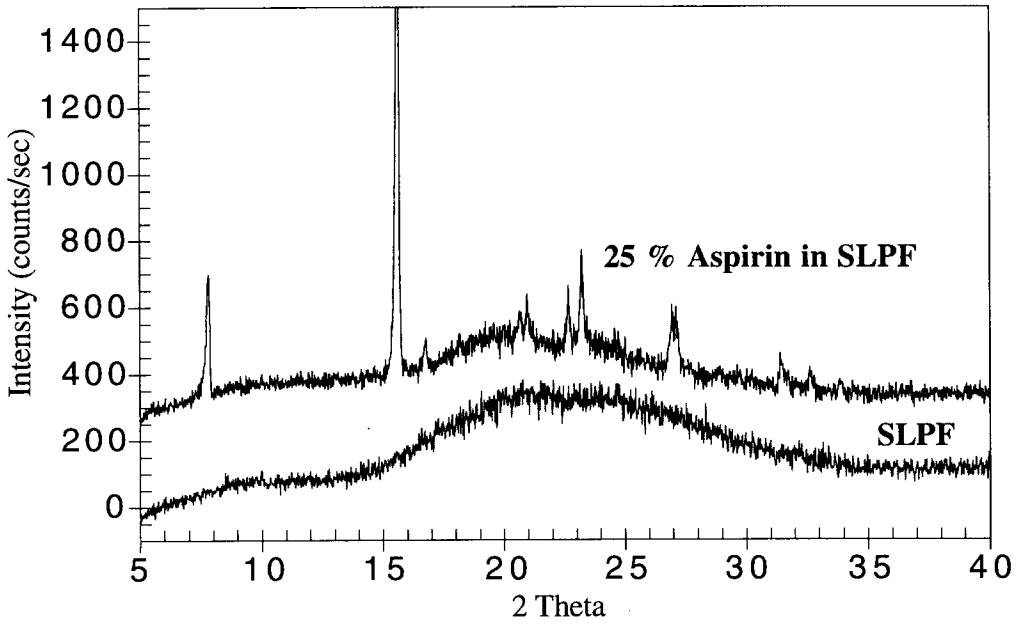

Figure 6 WAXS of cast SLPF and aspirin/SLPF blended thin films. Peaks from crystalline aspirin appear in the blended thin film, indicating phase separation.

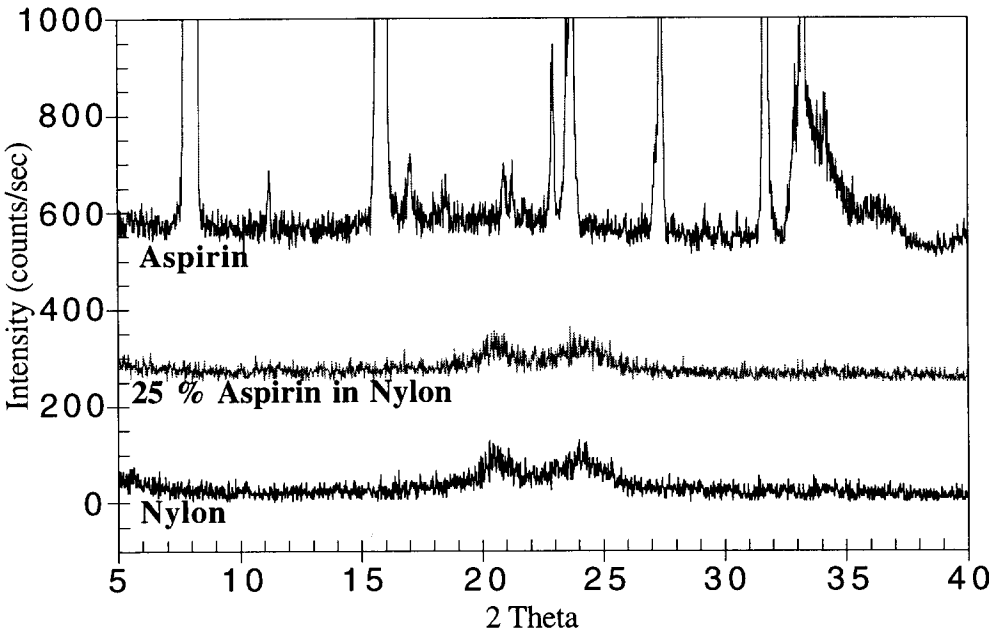

Figure 7 WAXS of electrospun aspirin, nylon, and blended aspirin/nylon thin films. Crystalline aspirin peaks are absent in the blended thin film.

For molecules that will incorporate in the solid polymer coating, it was necessary to determine the homogeneity of the blend. The SEM observations of aspirin/SLPF gave no evidence for inhomogeneous blending, but did not conclusively show that the blend is homogeneous. Given the ability of aspirin and other small molecules to readily crystallize, the best evidence for inhomogeneous mixing came from the WAXS data. In both the aspirin/nylon (Figure 5) and the aspirin/SLPF (Figure 6) systems, the films that are cast from solution instead of electrospun onto surface revealed crystalline domains. These films took about an hour to dry, and in that time, the blended components phase separate and crystallize. The fibers that made up the electrospun coatings dried in seconds, which apparently prevented crystalline domains from forming. Although

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it was not possible to collect WAXS data from electrospun aspirin/SLPF films, the lack of crystalline aspirin diffraction in TEM specimens indicated that there were no crystalline domains in the electrospun aspirin/SLPF films.

The examination of the aspirin/SLPF films after soaking supported the idea that the films were homogeneously blended. Although it was confirmed by precipitation that aspirin did diffuse out of the film, the lack of detectable morphological change showed that the aspirin did not diffuse out from large pores, which could indicate aspirin rich domains. Large pores did exist in the kanamycin/SLPF fibrous coatings that had soaked in water for 3 days, but conclusive evidence for kanamycin rich domains has not been found.

The morphology of the coating was seen to change with the addition of more small molecules. The fibrous nature of the coating gave way to droplets due to a decrease in the interactions among the polymer chains that make fiber formation possible. The differences in molecular weight among the included molecules did not cause systematic changes in the morphology of the fibers. Molecules as large as 1.4 kD did not disrupt the fibrous structure when included at 25% by weight.

CONCLUSIONS AND FUTURE WORK

The morphology of these coatings changes when the concentration of small molecule exceeds 50% by weight, and the fibrous morphology persists at lower concentrations. While solvent casting of small molecules with polymers can often produce inhomogeneous blends [11], the electrospinning process allows for the solution processing of small molecules (soluble in formic acid) blended with polymers into homogeneous fibrous coatings. Larger molecules that readily crystallize need to be examined using the same techniques to determine the influence of molecular weight on blend homogeneity.

Since these blended coatings are processed from solution, it is necessary to determine the effect of the processing solvent on the biological activity of the included molecules. Preliminary experiments with kanamycin indicate that it is still effective after processing. Larger, neurite specific proteins need to be evaluated both for the response to processing and for their effect on the structure of the coating.

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EDUCATION

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Thesis title: "Effect of a Porous Collagen-Glycosaminoglycan on the Early Healing of Tendon in a Novel Animal Model"
Advisors: Professor I.V. Yannas (M.I.T.) and Professor M. Spector (Harvard Medical School)

University of California at Berkeley, Berkeley, CA
B.S., Materials Science and Engineering (May 1990)

EXPERIENCE

University of Michigan, Department of Materials Science and Engineering, Ann Arbor, MI
Post-doctoral Fellow: In vitro investigation of the behavior of neuronal and glial cells on patterned substrates of ECM proteins and genetically-engineered proteins. Development, manufacturing and charaterization of polysaccharides hollow membrane fibers. (November 1996-present)

Massachusetts Institute of Technology, MSE Dept., Cambridge, MA
Brigham and Women's Hospital, Orthopedic Research Laboratory, Boston, MA
Research Assistant: Developed novel animal model to study *in situ* healing of Achilles tendon. Manufactured and characterized porous collagen-GAG copolymer matrices. Assisted in development and implementation of surgical protocol of the animal model. Introduced and implemented histological and immunohistochemical techniques and characterization to laboratory. Developed histological and immunohistochemical characterization and quantification of tendon tissue and neurological tissue such as sciatic nerve and spinal cord tissue. Utilized ultrastructural characterization techniques to quantify tendon tissue. Developed tissue culture and cell seeding protocol for project. (July 1991-September 1996)

IBM Almaden Research Center, Advanced Recording Head Development, San Jose, CA
Co-op Student: Optimized the geometrical configuration of multi-layer magnetic thin films for high speed disk drives. Refined experimental techniques to study the hysteresis behavior of magnetic thin films as a function of temperature. (June-December 1989)

Lawrence Berkeley Laboratory, MSCD Dept., Berkeley, CA
Student Assistant: Developed specimen configuration to investigate thermal fatigue properties of lead-tin solders for use in electronic packaging industry. (May 1988- May 1989)

AWARDS

Program in Polymer Science and Technology (PPST) fellowship (Fall 1990)

1989's Recipient of the Elaine C. Shen Memorial Gift Award for Materials Science majors.

PUBLICATIONS/ PRESENTATIONS

L. K. Louie, D. Schulz-Torres, L. Sullivan, I. V. Yannas, M. Spector, "Behavior of Fibroblasts Cultured in Porous Collagen-GAG Copolymer Matrices", Accepted for oral presentation at the 1997 Annual Meeting of the Society for Biomaterials, New Orleans, LA, April 30-May 4, 1997.

L. K. Louie, I. V. Yannas, H.-P. Hsu, M. Spector, "Effect of a Collagen-GAG Matrix on Early Healing in a Novel Rabbit Model", Poster Presentation at the 43rd Annual Meeting of the Orthopaedic Research Society, San Francisco, CA, February 9-13, 1997.

S. Nehrer, H. H. Breinan, A. Ramappa, G. Young, S. Shortkroff, L. Louie, C. B. Sledge, I. V. Yannas, M. Spector, "Pore Characteristics and Collagen Type Influence Behavior of Canine Chondrocytes Seeded in Matrices", *Biomaterials*

L. K. Louie, I. V. Yannas, M. Spector, "Tendon", in preparation.

L. K. Louie, I. V. Yannas, H.-P. Hsu, M. Spector, " Early Healing of Tendon Defects Implanted with a Porous Collagen-GAG Matrix: A Histological Evaluation", in submission.

L. K. Louie, I. V. Yannas, H.-P. Hsu, M. Spector, " Early Healing of Tendon Defects Implanted with a Porous Collagen-GAG Matrix: A Histological and Ultrastructural Evaluation", Poster Presentation at the Tissue Engineering Society, Orlando, FL, December 13-15, 1996.

L. K. Louie, H.-P. Hsu, I. V. Yannas, M. Spector, "The Effect of a Porous Collagen-GAG Matrix on Tendon Healing", Poster Presentation at the 1995 Annual Meeting of the Biomedical Engineering Society, Boston, MA, October 6- 8, 1995.

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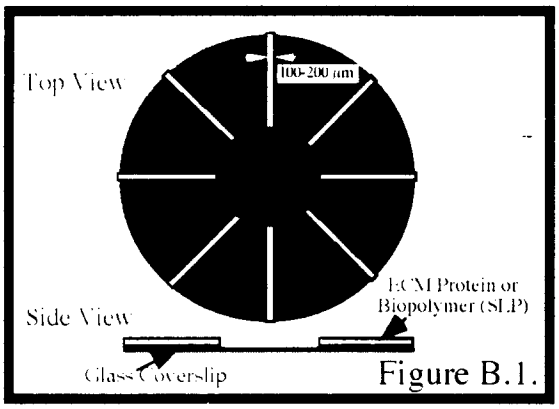

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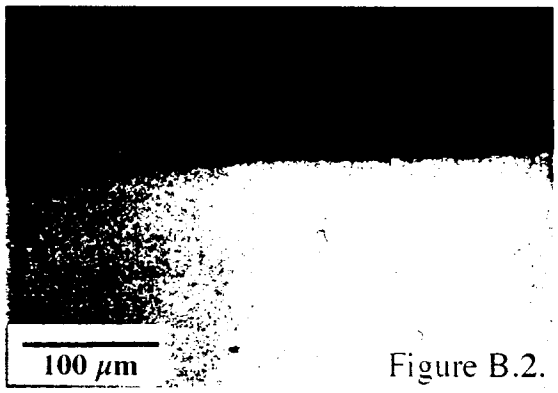

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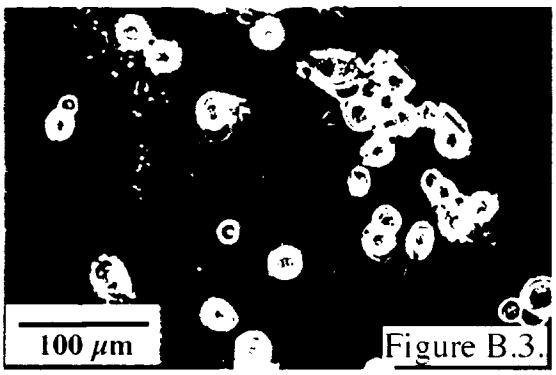

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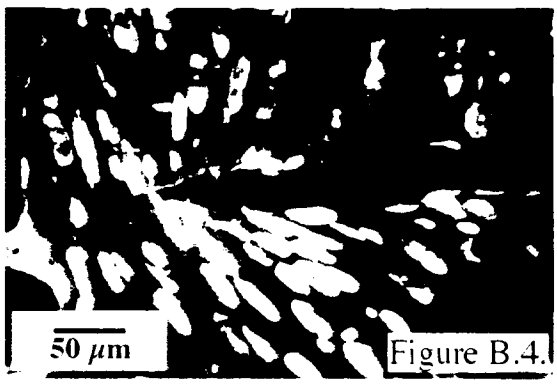

Figure B.4.